IN THE SPECIFICATION

Please replace the paragraph on page 1, lines 7-13 with the following replacement paragraph.

The present invention relates to a pretreatment kit for saliva for identification and quantitatively determining streptococci mutans, as one of cariogenic bacteria in human saliva, by immunochromatography utilizing an antigen-antibody reaction, and a pretreatment method for saliva using the pretreatment kit for saliva.

Please replace the paragraph on page 1, lines 15-23 with the following replacement paragraph.

It has been known that there is close relation between the presence of streptococci mutans and the generation of dental caries in a human mouth, and therefore, the morbidity risk and the current condition of morbidity can be comprehended to provide benefits to quite a number of people if the presence or absence and the amount of streptococci mutans in a human mouth can be conveniently examined.

Please replace the paragraphs starting on page 3, line 11 and ending on page 6, line 2 with the following replacement paragraphs.

It seems that identification and quantitative determination of streptococci mutans in the human mouth can be carried out by applying the technique, but it has not been put into practical use because of the following problems. That is, it is necessary that a sample used for the immunochromatography technique pass through a porous membrane by the capillary phenomenon. However, because the major sample applied to the examination of bacteria in the mouth, such as streptococci mutans, is saliva, a high viscosity substance present in saliva, which is referred to as mucin, clogs the pores of the porous membrane. Furthermore, because

mucin has such a function that aggregates epithelial attachment cells stripped off from oral mucosa, the pores of the porous membrane are clogged with these substances to inhibit transmission of streptococci mutans.

In addition to mucin, there is another problem complicating identification and quantitative determination of streptococci mutans by the immunochromatography technique. That is, the streptococci mutans to be measured is a bacterium having a diameter of about 1 μm solely but often forms a chain with 10 to 20 or more bacteria owing to the nature of streptococci, which may be a factor of inhibiting migration in the porous membrane. Furthermore, the streptococci mutans forms glucan having adherence from sucrose in foods and is often severely aggregated. The chain formation and aggregation of streptococci mutans cause clogging in the porous membrane and also reduce the surface area of the streptococci to affect quantitative determination of the number of antigens present on the surface of the streptococci mutans, which reduces accuracy of the measurement.

In the immunochromatography technique, detection of an analytic object is generally carried out by using two antibodies. The first antibody is retained in a porous membrane formed with glass fibers or the like on the side where a sample is dropped, and the antibody is generally labeled with latex particles, gold colloid particles or the like (hereinafter, sometimes referred to as a labeled antibody). In the case where the analytic object to be measured is present in the sample, when passing the sample through the porous membrane, the labeled antibody recognizes the analytic object to be measured and is bonded thereto. The composite of the analytic object and the labeled antibody is flowed by capillary phenomenon toward another porous membrane having the second antibody (hereinafter, sometimes referred to as a trap antibody) immobilized thereon, for example, in the form of strips, and the complex of the analytic object and the labeled antibody is recognized, trapped and detected as a visible signal (in the form of strips in this case). In the case where the

immunochromatography technique is applied to saliva as a sample, however, the composite of a labeled antibody and streptococci mutans is trapped in the membrane retaining the labeled antibody but does not efficiently flow by capillary phenomenon toward the porous membrane having the trap antibody immobilized therein to cause such a problem that the accuracy of the measurement is reduced.

Please replace the paragraph on page 6, lines 4-17 with the following replacement paragraph.

An object of the invention is to solve the problems associated with the conventional technique for identification and quantitative determination of streptococci mutans, as one of cariogenic bacteria in human saliva, by immunochromatography utilizing an antigen-antibody reaction, and to provide a pretreatment kit for saliva and a pretreatment method for saliva in that aggregation caused by mucin and chain formation of streptococci mutans in saliva can be removed in a simple operation, and a complex of a labeled antibody and streptococci mutans effectively flows out from a porous membrane retaining the labeled antibody.

Please replace the paragraphs beginning on page 6, line 23 and ending on page 7, line 14 with the following replacement paragraphs.

(1) Mucin and glucan in saliva are dissolved to act on an adventitia of streptococci mutans to suppress aggregation.

(2) Upon using a particular surface active agent in addition thereto, a protein in streptococci mutans is solubilized, whereby the streptococci mutans is efficiently flowed through the porous membrane.

(3) Upon using a particular metallic salt, i.e., sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate or manganese sulfate, in addition thereto, the complex of a labeled antibody and streptococci mutans can be efficiently flowed out from the membrane retaining the labeled antibody.

Please replace the paragraphs beginning on page 9, line 23 and ending on page 12, line 2 with the following replacement paragraphs.

A pretreatment method for saliva in identification and quantitative determination of streptococci mutans by immunochromatography using the pretreatment kit for saliva of the present invention contains, as one aspect, steps of mixing at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and manganese sulfate with at least one of (A) an aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L, (B) an aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L, and (C) a nonionic surface active agent and/or an amphoteric surface active agent, in an amount of 5 to 25% by weight; and mixing the components (A), (B) and (C) by dropping in an arbitrary order (hereinafter, referred to as a first method).

The pretreatment method for saliva in identification and quantitative determination of streptococci mutans by immunochromatography also contains, as another aspect, steps of mixing at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and manganese sulfate with at least one of (A) an aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L and (B) an aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L, in an amount of 5 to 25% by weight; mixing (C) a nonionic surface active agent and/or an amphoteric surface active agent in at least one

of the components (A) and (B); and mixing the components (A) and (B) by dropping in an arbitrary order (hereinafter, referred to as a second method).

The pretreatment method for saliva in identification and quantitative determination of streptococci mutans by immunochromatography also contains, as another aspect, steps of mixing at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and manganese sulfate with at least one of (A) an aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L, (B) an aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L, (C) a nonionic surface active agent and/or an amphoteric surface active agent, and (D) tris(hydroxymethyl)aminomethane, in an amount of 5 to 25% by weight; and mixing the components (A), (B), (C) and (D) by dropping in such an order that the component (A) is in contact with the component (B) in the presence of the component (D) (hereinafter, referred to as a third method).

Please replace the paragraphs beginning on page 12, line 18 and ending on page 16, line 13 with the following replacement paragraphs.

The aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L as the component (A) used in the pretreatment kit for saliva and the pretreatment method for saliva according to the present invention exerts a function to act on mucin in saliva and glucan present on an adventitia of streptococci mutans to suppress aggregation of streptococci mutans, so as to facilitate migration of the streptococci mutans as an antigen in the porous membrane. The use of sodium hydroxide as an alkali solution is essential, but sodium carbonate, disodium hydrogen phosphate and the like are not suitable, and the examination of streptococci mutans cannot be carried out with other alkali solutions than sodium hydroxide. It is supposed that this is because an alkali treatment other than sodium hydroxide may impair

the structure of the antigen of the streptococci mutans. The concentration of sodium hydroxide in the aqueous solution is necessarily 0.01 to 10 mol/L, and a concentration less than 0.01 mol/L cannot provide sufficient effect, whereas that exceeding 10 mol/L breaks the antigen of the streptococci mutans to deteriorate the detection accuracy.

The aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L as the component (B) used in the pretreatment kit for saliva and the pretreatment method for saliva according to the present invention exerts a function to suppress the chain formation of the streptococci mutans, so as to facilitate migration of the streptococci mutans as an antigen in the porous membrane. The use of tartaric acid and/or citric acid as an acid is essential, but other acids, such as hydrochloric acid, sulfuric acid, nitric acid, acetic acid, lactic acid and maleic acid, are not suitable, and the objective accuracy in examination cannot be obtained even when the other acids are used in combination with sodium hydroxide. It is supposed that this is because the other acids than tartaric acid and citric acid may impair the structure of the antigen of the streptococci mutans. The concentration of tartaric acid and/or citric acid in the aqueous solution is necessarily 0.01 to 3 mol/L, and a concentration less than 0.01 mol/L cannot provide sufficient effect, whereas that exceeding 3 mol/L is not suitable because the solubility of tartaric acid and/or citric acid comes to the limit to form precipitation.

The nonionic surface active agent and/or the amphoteric surface active agent as the component (C) used in the pretreatment kit for saliva and the pretreatment method for saliva according to the present invention exerts a function to solubilize a protein present on the surface of streptococci mutans, so as to facilitate efficient flow of the streptococci mutans through the porous membrane. An ionic surface active agent has been often used in immunochromatography for facilitating smooth migration of a sample solution or an antigen solution within an examination apparatus. However, the surface active agent used in the

pretreatment kit for saliva and the pretreatment method for saliva used for identification and quantitative determination of streptococci mutans according to the present invention is necessarily a nonionic surface active agent and/or an amphoteric surface active agent as a result of experimentation, but detection of an antigen with an antibody cannot be attained with an anionic surface active agent, such as sodium lauryl sulfate and sodium dodecylbenzenesulfonate.

The surface active agent used the present invention may be any nonionic surface active agent and/or amphoteric surface active agent without particular limitation, and any of those that are generally used as a solubilizing agent for a membrane protein can be used. The detection sensitivity of streptococci mutans varies depending on the species of the nonionic surface active agent and/or the amphoteric surface active agent used, and it is preferred to use one kind or a mixture of two or more kinds selected from the group consisting of polyethylene glycol monooctylphenyl ether, n-octyl-β-D-glucoside, n-heptyl-β-D-thioglucoside and n-octyl-β-D-thioglucoside as the nonionic surface active agent, and one kind or a mixture of two or more kinds selected from the group consisting of CHAPS (3-((3-cholamide-propyl)-dimethylammonio)-1-propanesulfonate) and CHAPSO (3-((3-cholamide-propyl)-dimethylammonio)-1-hydroxypropanesulfonate) as amphoteric surface active agent, from the standpoint of detection sensitivity.

Please replace the paragraphs beginning on page 17, line 19 and ending on page 19, line 13 with the following replacement paragraphs.

In the pretreatment kit for saliva and the pretreatment method for saliva according to the present invention, at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and

manganese sulfate is contained in at least one of the aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L (A), the aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L (B) and the nonionic surface active agent and/or the amphoteric surface active agent (C), in an amount of 5 to 25% by weight in order to have the complex of the labeled antibody and streptococci mutans flown out from the membrane retaining the labeled antibody efficiently. The at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and manganese sulfate exerts a function to aggregate various kinds of proteins present in saliva by salting out and counteract the mutual action between the labeled antibody and the membrane retaining the same, so as to provide by the function an effect of facilitating efficient flow of the complex of the labeled antibody and the streptococci mutans from the membrane retaining the labeled antibody.

The at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and manganese sulfate is necessarily contained in at least one of the aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L (A), the aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L (B) and the nonionic surface active agent and/or the amphoteric surface active agent (C), in an amount of 5 to 25% by weight. In the case where the amount is less than 5% by weight, the effect cannot be sufficiently obtained, and the complex of the labeled antibody and the streptococci mutans cannot be efficiently flowed out from the membrane retaining the labeled antibody. In the case where it exceeds 25% by weight, on the other hand, the detection accuracy is rather deteriorated.

Please replace the paragraph beginning on page 20, line 15 and ending on page 21, line 16 with the following replacement paragraph.

In the first method according to the present invention, upon identification and quantitative determination of streptococci mutans by immunochromatography, at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate and manganese sulfate is mixed with at least one of the components (A), (B) and (C), in an amount of 5 to 25% by weight, and then the aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L (A), the aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L (B) and the nonionic surface active agent and/or the amphoteric surface active agent (C) are mixed by dropping in an arbitrary order. In order to simplify the operation in the second method according to the present invention, the nonionic surface active agent and/or the amphoteric surface active agent (C) is mixed with at least one of the aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L (A) and the aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L (B), and then the aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L (A) and the aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L (B) are mixed by dropping in an arbitrary order.

Please replace the paragraph beginning on page 22, line 11 and ending on page 23, line 9 with the following replacement paragraph.

In addition to the first and second methods, in the third method according to the present invention, upon identification and quantitative determination of streptococci mutans by immunochromatography, at least one substance selected from the group consisting of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium

sulfate and manganese sulfate is mixed with at least one of the aqueous solution of sodium hydroxide having a concentration of 0.01 to 10 mol/L (A), the aqueous solution of tartaric acid and/or citric acid having a concentration of 0.01 to 3 mol/L (B), the nonionic surface active agent and/or an amphoteric surface active agent (C), and the tris(hydroxymethyl)aminomethane (D), in an amount of 5 to 25% by weight, and then the components (A), (B), (C) and (D) are mixed by dropping in such an order that the component (A) is in contact with the component (B) in the presence of the component (D). It is preferred in this case that the component (A), (B) and (D), at least one of which is mixed with the component (C), are mixed by dropping in such an order that the component (A) is in contact with the component (B) in the presence of the component (D).

Please replace the paragraph on page 23, line 21 and ending on page 24, line 10 with the following replacement paragraph.

The sample of saliva having been treated by the pretreatment kit for saliva and the pretreatment method for saliva according to the present invention can be subjected to identification and quantitative determination of streptococci mutans by an antigen-antibody reaction using the conventional immunochromatography technique. The antibody can be obtained by an ordinarily employed way. For example, it may be obtained by the establishment of hybridoma by cell fusion according to Kohler and Milstein (Kohler G, C. Milstein, Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, vol. 256, p. 495-497 (1975)), or may be those simply purified from a serum of an animal having been immunized with the antigen.

Please replace the paragraphs beginning on page 27, line 7 and ending on page 28, line 9 with the following replacement paragraphs.

(2) Quantitative Determination of Streptococci mutans

The results of quantitative determination of streptococci mutans in saliva by the immunochromatography using the foregoing reagents and device was compared with the number of streptococci mutans in saliva by the conventional method of calculation from the number of colonies.

1. Examination Method by Immunochromatography

The reactivity between the streptococci mutans and the antibody immobilized on the antibody-immobilized porous membrane was detected by the following principle. Upon passing saliva through the labelled antibody retention of the antibody-immobilized porous membrane strip, the labelled antibody was bonded to the streptococci mutans in saliva to color in red. The complex of the streptococci mutans and the labelled antibody migrated in the antibody-immobilized porous membrane strip, and it was then trapped by the antibody (trap antibody) immobilized, for example, in the form of strips, in the antibody-immobilized porous membrane strip and thus confirmed as a strip-shape mark.

2. Number of Streptococci mutans by Conventional Calculation from Number of Colonies

Please replace the paragraphs beginning on page 55, line 1 and ending on page 56, line 7 with the following replacement paragraphs.

It was confirmed from the foregoing results that the pretreatment method for saliva by using the pretreatment kit for saliva according to the present invention could remove aggregation caused by mucin and chain formation of streptococci mutans in saliva, and since the amount of the complex of the labelled antibody and the streptococci mutans remaining in

the labelled antibody retention was small, the effect of preventing such a phenomenon that the complex of the labelled antibody and the streptococci mutans remained in the membrane retaining the labelled antibody was confirmed.

As having been described in detail, upon identification and quantitative determination of streptococci mutans, which is a type of cariogenic bacteria in human saliva, by immunochromatography utilizing an antigen-antibody reaction, the pretreatment kit for saliva and the pretreatment method for saliva according to the present invention can remove aggregation caused by mucin and chain formation of streptococci mutans in saliva in a simple operation, and can efficiently flow a complex of a labeled antibody and streptococci mutans from a porous membrane retaining the labeled antibody, whereby identification and quantitative determination can be carried out with high accuracy. Therefore, it provides significant value by contributing to the field of dentistry.